
# Epidemiologic Foundation for the Assessment of Genetic Tests

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# Genetic test - definition

"the analysis of human DNA, RNA, chromosomes, proteins, and certain metabolites in order to detect heritable disease-related genotypes,mutations, phenotypes, or karyotypes for clinical purposes."

Holtzman & Watson, 1999

# Potential applications

- Diagnosis
- Primary screening in general population
- Triage of individuals at high-risk

# Evaluation

- Analytic validity
- Clinical validity
- Clinical utility

# Clinical validity

the accuracy with which a test predicts a clinical outcome

the sensitivity, specificity, and predictive value of a test in relation to a particular phenotype

Holtzman & Watson, 1999

# Clinical validity

- When a test is used diagnostically, clinical validity measures the association of the test with the current existence of that disorder.
- When a test is used to identify genetic susceptibility, as in genetic screening, clinical validity measures the accuracy with which it predicts a future clinical outcome.

Pinsky et al., 2004

# Clinical validity

| | | **Disease** | |
|---|---|---|---|
| | | + | - |
| **Test** | + | True positive (TP) | False positive (FP) |
| | - | False negative (FN) | True negative (TN) |
| | | All with disease (TP+FN) | All without disease (TN+FP) |
| | | **Sensitivity**= TP/(TP+FN) | **Specificity**= TN/(TN+FP) |

# Clinical validity

| | | Disease | | |
|---|---|---|---|---|
| | | + | - | |
| Test | + | True positive (TP) **9900** | False positive (FP) **900** | |
| | - | False negative (FN) **100** | True negative (TN) **89,100** | |
| | | All with disease (TP+FN) **10,000** | All without disease (TN+FP) **90,000** | All subjects (TP+FP+TN+FN) **100,000** |
| | | Sensitivity= TP/(TP+FN) **99%** | Specificity= TN/(TN+FP) **99%** | Prevalence (TP+FN)/ (TP+FP+TN+FN) **10%** (9900+100)/100,000 |

# Clinical validity

| | | Disease | | |
|---|---|---|---|---|
| | | + | - | |
| Test | + | True positive (TP) **9900** | False positive (FP) **900** | |
| | - | False negative (FN) **100** | True negative (TN) **89,100** | |
| | | All with disease (TP+FN) **10,000** | All without disease (TN+FP) **90,000** | All subjects (TP+FP+TN+FN) **100,000** |
| | | Sensitivity= TP/(TP+FN) **99%** | Specificity= TN/(TN+FP) **99%** | Prevalence (TP+FN)/ (TP+FP+TN+FN) **10%** (9900+100)/100,000 |

| | | Disease | | |
|---|---|---|---|---|
| | | + | - | |
| Test | + | True positive (TP) **990** | False positive (FP) **990** | |
| | - | False negative (FN) **10** | True negative (TN) **98,010** | |
| | | All with disease (TP+FN) **1000** | All without disease (TN+FP) **99,000** | All subjects (TP+FP+TN+FN) **100,000** |
| | | Sensitivity= TP/(TP+FN) **99%** | Specificity= TN/(TN+FP) **99%** | Prevalence (TP+FN)/ (TP+FP+TN+FN) **1%** (990+10)/100,000 |

# Clinical validity

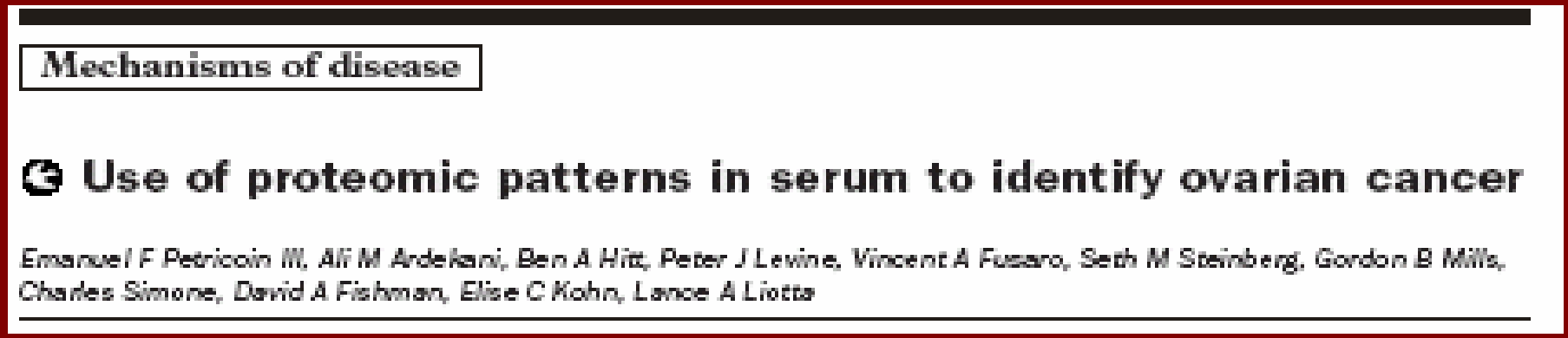
| | | Disease | | | |
|---|---|---|---|---|---|
| | | + | - | | |
| Test | + | True positive (TP) | False positive (FP) | All test positive (TP+FP) | **PPV =** TP/(TP+FP) |
| | - | False negative (FN) | True negative (TN) | All test negative (TN+FN) | **NPV=** TN/(TN+FN) |
| | | All with disease (TP+FN) | All without disease (TN+FP) | All subjects (TP+TN+FP+FN) | |
| | | Sensitivity= TP/(TP+FN) | Specificity= TN/(TN+FP) | Prevalence (TP+FN)/ (TP+TN+FP+FN) | |

# Clinical validity

| | | Disease + | Disease - | |
|---|---|---|---|---|
| Test | + | True positive (TP) **9900** | False positive (FP) **900** | **PPV** = TP/(TP+FP) 9900/10800 = **91.7%** |
| | - | False negative (FN) **100** | True negative (TN) **89,100** | **NPV**= TN/(TN+FN) 89100/89200= **99.9%** |
| | | All with disease (TP+FN) **10,000** | All without disease (TN+FP) **90,000** | All subjects (TP+FP+TN+FN) **100,000** |
| | | Sensitivity= TP/(TP+FN) **99%** | Specificity= TN/(TN+FP) **99%** | Prevalence (TP+FN)/ (TP+FP+TN+FN) **10%** (9900+100)/100,000 |

| | | Disease + | Disease - | |
|---|---|---|---|---|
| Test | + | True positive (TP) **990** | False positive (FP) **990** | **PPV** = TP/(TP+FP) 990/1980 = **50%** |
| | - | False negative (FN) **10** | True negative (TN) **98,010** | **NPV**=TN/(TN+FN) 98010/98020 = **100%** |
| | | All with disease (TP+FN) **1000** | All without disease (TN+FP) **99,000** | All subjects (TP+FP+TN+FN) **100,000** |
| | | Sensitivity= TP/(TP+FN) **99%** | Specificity= TN/(TN+FP) **99%** | Prevalence (TP+FN)/ (TP+FP+TN+FN) **1%** (990+10)/100,000 |

# Clinical validity



Mechanisms of disease

## Use of proteomic patterns in serum to identify ovarian cancer

*Emanuel F Petricoin III, Ali M Ardekani, Ben A Hitt, Peter J Levine, Vincent A Fusaro, Seth M Steinberg, Gordon B Mills, Charles Simone, David A Fishman, Elise C Kohn, Lance A Liotta*

| | |
|---|---|
| **Sensitivity** | **100%** |
| **Specificity** | **95%** |
| **Positive predictive value** | **94%** |

**“These findings justify a prospective population-based assessment of proteomic pattern technology as a screening tool for all stages of ovarian cancer in high-risk and general populations.”**

# Clinical validity

Study base: 50 women with ovarian cancer, 66 from unaffected women or those with non-malignant disorders

More typical study base: in 1601 women referred because of family history, 11 cases of ovarian cancer diagnosed over 42 months
(Bourne et al., 1993)

TP = 11

TN = (1601-11)=1590

FP= 1590*0.05 = 80

PPV = TP/(TP+FP) = 11/(80+11) = **12%**

# Parameters of clinical utility are related to genotype frequency (g), disease frequency (p) and relative risk (R)

| Genotype | Will develop disease | Will not develop disease | Total |
|---|---|---|---|
| + | sens*p | (1-spec)*(1-p) | g |
| - | (1-sens)*p | spec*(1-p) | 1-g |
| Total | p | (1-p) | 1 |

e.g. Sensitivity = R.g/ (1 + g.(R-1)

Khoury et al., 1993
Yang et al., 2000

# Genetic markers for COPD

| Genetic marker | G | R | Sensitivity (%) | Specificity (%) | PPV |
|---|---|---|---|---|---|
| **Homozygosity for PiZ** | .0005 | 20 | 1.0 | 99.99 | 99.1 |
| **ABH nonsecretor** | .25 | 1.5 | 33.3 | 75.4 | 6.7 |
| **Blood group A antigen** | .45 | 1.3 | 51.5 | 55.3 | 5.7 |

Khoury et al., 1993

# Clinical utility, genotype frequency, disease frequency and relative risk

- Even when RRs are high, sensitivity and PPV are affected by the relative magnitude of disease and genetic marker frequencies.
- When the genetic marker is less frequent than the disease, PPV increases with increasing RR but sensitivity remains low.
- When the genetic marker is more frequent than the disease, sensitivity increases with increasing RR but PPV remains low.
- When marker and disease frequencies are equal, both PPV and sensitivity increase with increasing RR.

# Issues in determining clinical validity

| Issue | RR | Genotype frequency | Disease frequency |
|---|---|---|---|
| Study design | ✓ | ✓ & external data | External data |
| Selection bias | ✓ | ✓ | If not population-based |
| Statistical power | ✓ | Precision? | Precision? |
| Publication bias | ✓ | ? | ? |
| G-E interaction | ✓ | | |
| Information bias (G – analytic validity) | ✓ (G & E) | ✓ (G) | |
| Confounding | population stratification, LD, other | | |

# Risk of breast cancer in *BRCA1/BRCA2* mutation carriers at age 70

| Study | Population | Gene(s) | **Risk (%) by age 70 (95%CI)** |
|---|---|---|---|
| ***Large high-risk families*** | | | |
| Ford et al. 1994 | IBCLC multicase families; 33 families | *BRCA1* | **87 (72-95)** |
| Easton et al. 1997 | 2 *BRCA2* families | *BRCA2* | **80 (29-98)** |
| ***Relatives of cases from population-based case-control studies or of cases from consecutive series of newly incident cases*** | | | |
| Struewing et al., 1997 | Ashkenazi Jews, Washington DC, recruited by media – 1st degree rels of 27 cases | *BRCA1/ BRCA2* [known founder mutations] | **56 (40-73)** |
| Hopper et al., 1999 | Australia, young probands – 1st degree rels of 18 cases | *BRCA1/ BRCA2* [extensive sequencing] | **40 (15-65)** |
| Antoniou et al., 2000 | UK,– entire pedigrees of 12 cases | *BRCA1* [extensive sequencing] | **45 (22-76)** |
| ***Family data not used*** | | | |
| Satapogan et al., 2001 | Ashkenazi Jews – 79 hospital based cases, and 62 controls | *BRCA1*<br>*BRCA2* | **46 (31-80)**<br>**26 (14-50)** |

# Clinical utility

the net value of the information gained from a genetic test in changing disease outcomes

Gwinn 2004

# Observational evidence & randomized control trials (RCTs)

- Differences in estimated magnitude of treatment effect between RCTs and observational studies are very common
- The directions of the differences are difficult to predict

(Britton et al., 1998; MacLehose et al., 2000; Ioannidis et al., 2001)